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Effects of Nitrate and Ammonium Nitrogen Sources on the Seedling Growth and Root Phosphoenolpyruvate Carboxylase Activity of Different Crop Species[1]

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Abstacts: Seven crop species, including maize, sorghum, rice, wheat, barley, peanut and soybean, were grown hydroponically with either nitrate or ammonium N at a concentration of 150 ppm. Rates of seedling growth were determined to study the effect of N sources on the performances of carbon metabolism. As ammonium N could enhance the activity of PEP carboxylase in the roots of many crops, attempts had been made to clarify the characteristics of this enzyme in order to provide basis for further studies. Experimental results showed that crops grew better in nitrate N than in ammonium N medium. However, shoots of rice and wheat gained more fresh weight when treated with ammonium N which was different from the results on maize, sorghum and peanut, indicating difference in response to N sources among plant species. Ammonium N could enhance the activity of PEP carboxylase of various crops. The increase was 8—61% in the leaf blade and as high as 4.5 folds in the root. PEP carboxylase extracted from the roots of Tainung 67 rice with the methods employed in this experiment was extremely stable. No decrease in enzyme activity was observed up to 7 hrs after extraction. Optimal pH for rice root PEP carboxylase was between 8.0 and 8.5 with either Tris-HCl (100 mM) or Hepes-KOH(50 mM) buffer. Kinetic study showed that the response curves of velocity versus substrate (PEP) concentration was hyperbolic at pH 8.0 and sigmoid at pH 7.0. Reciprocal plots of the data was linear, indicatng Michaelis-Menten kinetics. Km value for PEP was calculated to be 0.16 mM at pH 8.0. Preliminary results suggested that the ammonium-N responsive root PEP carboxylase was similar to the same enzyme from leaf blades of C3 plants in many aspects. As PEP plays a very important role in carbon metabolism, and in view that ammonium-N at a very low concentration (e. g., 1 ppm) could enhance significantly the activity of root PEP carboxylase, the finding of this experiment could be utilized for further study on the interaction of N and carbon metabolism in plants.

Introduction

It is generally recognized that although plant may take up nitrogen in various forms, the main sources in natural conditions are nitrate and ammonium ions[12]. The physiological responses of plants to these ions are very different[1] and the ability of

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plants to absorb and metabolize them varies greatly [4,8,9]. It has been well noticed that the source and availability of nitrogen can affect carbon metabolism in the plants [4,7]. Its implication on plant productivity has also been assessed [2]. Many research projects focused on the influences of nitrate and ammonium nutrition on photosynthesis and related reactions have been executed and published in recent years. However, the growth responses to different nitrogen sources vary considerably according to plant species and plant age [21,24], and the interactions between nitrogen and carbon metabolism remain obscure [7,20]. The initial aim of this study was to examine the influences of nitrate and ammonium nitrogen on the growth of seedlings of various crops under controlled environment and to compare the resulted changes in photosynthetic and respiratory activities. During the course of this study, it was found that the activity of PEP carboxylase, the key enzyme for photosynthesis in C4 plants, increased markedly in the roots as a result of ammonium-N influences. Hence, efforts had been made to study the characteristics of this enzyme under the experimental conditions employed.

PEP plays a very important role in plant metabolism. For example, it can bind to at least six enzymes which catalyze the allylic replacement of phosphate [22]. PEP is also the control points of glycolysis, gluconeogenesis and in the control of cellular pH [3]. It is therefore worthy to understand the possible importance of PEP as a function of the interrelationship between nitrogen and carbon. As for the enzyme PEP carboxylase, it occurs in all plants and plays particularly important roles in carbon fixation in C4 plants and in CAM plants. However, it has also been reported that PEP carboxylase extracted from the roots of several C4 plants was distinct from the leaf form [17,26]. Hence, the characteristic of this enzyme extracted from roots of C3 plants, especially rice, under ammonium nutrition was studied with the purpose of providing information to future investigation related to this aspect.

Materials and Methods

Plant species used in this experiment included a japonica rice (*Oryza sativa* L.) variety, Tainung 67, two indica rice varieties, Taichung sen 3 and Pegonil (the latter has 1,000-grain-weight ca. 40g), a wheat (*Triticum aestivum* L.) variety, Taichung S. 2, a six-row barley (*Hordeum vulgare* L.) variety introduced from CIMMYT, CB-1, a single-cross maize (*Zea mays* L.) variety, Tainung 351, a single-cross sorghum (*Sorghum vulgare* Per.) variety, Taichung 3, a soybean (*Glycine max* (L.) Mer.) variety, Shih-Shih and a peanut (*Arachis hypogaea* L.) variety, Tainan S. 9. Rice, especially Tainung 67, was used extensively for the study of PEPC activities.

Seeds were soaked in aerated water for 12 hr (soybean and peanut), 24 hr (wheat and barley), 48 hr (maize and sorghum) or 72 hr (rice) followed by germinating in vermiculate for 7 to 10 days. Germination was kept in a growth chamber (Conviron PGW-36, Controlled Environments Co.) at day/night (14/10 hr) temperature of 28/20 C. Uniform seedlings were transplanted to plastic pots and grown hydroponically with the basic salts of Hoagland formulation. One or two seedlings were grown in a pot with 600 ml of culture solution. Nutrient solution was aerated vigorously by using a small compres-

sor connected via perforated polyethylene tubings and was renewed at a two-day interval. Plants were kept in the same growth chamber under a daily regime of 14-hr light at 28°C and 10-hr dark at 20°C. Illumination was provided with fluorescent and tungsten lamps giving 450 uE/m²/sec of irradiation at the top of the plants.

The culture solution contained the following essential elements with the specified concentrations (mg/l): K^+: 234, Ca^{2+}: 200, Mg^{2+}: 48, PO_4^{3-}: 95, SO_4^{2-}: 192, BO_3^{3-}: 2.9, Fe (Fe-EDTA): 2.2, Mn^{2+}: 0.27, Zn^{2+}: 0.13, Cu^{2+}: 0.13 and MnO_4^{2-}: 0.07. Nitrogen was supplied as either NO_3 or NH_4-N with a concentration of 150 ppm, unless otherwise specified. The pH of the freshly prepared solution was 4.85—4.90.

For the extraction of PEP carboxylase (EC 4.1.1.31), immediately after harvesting the leaves and roots under irradiation extracts were made in 4—5 volumes of buffer containing 100 mM Tris-HCl (pH 7.6), 20% (v/v) glycerol, 1 mM EDTA, 5 mM DTE, 1mM $MgCl_2$ and 2% (w/v) insoluble polyvinylpolypyrrolidone (PVP), using a chilled mortar and pestle. An aliquot was taken for chlorophyll determination prior to filtering through one layer of miracloth and centrifuging at 20,000 g for 10 min. PEP carboxylase was assayed spectrophotometrically at 30 C in a total volume of 1 ml by coupling oxaloacetae formation with malate dehydrogenase as described by Uedan and Sugiyama [27]. The assay mixture contained 50 mM Hepes-KOH, pH 8.0 (or 100 mM Tris-HCl, pH 8.0), 5 mM $MgCl_2$, 5 mM DTE, 0.2 mM NADH, 10 mM $NaHCO_3$, 2 mM PEP and 5 units of malic dehydrogenase. The reaction was initiated by the addition of PEP to the assay medium. A Hitachi 150—20 double beam spectrophotometer was used to record the decrease of absorbance at 340 nm.

Results

Vigorous growth of plants in the nitrate medium (initial pH 4.9) caused rapid drift towards alkalinity (pH 7 after two days of culture) in the culture solution, conversely with the ammonium medium plant growth caused an acid drift (pH 3.5;data not shown). The extent of pH drift was correlated with plant age and size. Older and larger plant, especially larger root system, caused more prominent pH drift than the younger and smaller plants. The drift occurred mainly in the first day after renewal of cultural solution.

Seedlings of all crop species tested grew better in the nitrate that in the ammonium culture solution in terms of fresh weight gain of the whole seedling (Table 1). The most significant case was found in maize where total fresh weight per seedling after 9 days in culture was 13.6 g for nitrate and 6.4 g for ammonium treatment, or a 2.14-fold difference was observed. The least difference was in rice where the fresh weight per seedling grown in nitrate solution was only 5—8% higher than that grown in ammonium solution.

It is worthy to note that for maize, sorghum, peanut and soybean, both the shoot and root gained more fresh weight when grown in nitrate than in ammonium medium (Table 1). For example, the ratio of fresh weight between nitrate and ammonium was 2.38 for shoot and 1.77 for root in maize. On the contrary, shoot of rice and wheat

Table 1. Fresh weight (mg/plant) of various crops under hydroponic culture as affected by different N sources[a]

Crop	Days in Culture	NO_3^-			NH_4^+		
		Shoot	Root	Total	Shoot	Root	Total
Corn	0	1,086± 59	793± 22	1,979± 46			
	9	9,311±257	4,330±126	13,641±209	3,922±214	2,440±186	6,362±199
	CGR[b]	914	393	1,307	315	183	498
Sorghum	0	314± 21	219± 34	533± 32			
	7	1,060±144	1,057±189	2,117±163	780±113	933±141	1,713±138
	CGR	107	120	227	67	102	169
Rice Tainung 67	0	77± 11	43± 13	120± 11			
	13	488± 51	442± 27	930± 41	561± 32	304± 28	865± 31
	CGR	32	31	63	37	20	57
Rice Pegonil	0	139± 27	119± 18	258± 23			
	13	945± 36	666± 42	1,611± 40	1,058± 49	477± 39	1,535± 46
	CGR	62	42	104	71	28	99
Wheat	0	205± 17	211± 27	416± 24			
	9	697± 22	764± 43	1,461± 39	783± 53	392± 33	1,175± 46
	CGR	54	61	115	64	20	84
Barley	0	224± 29	200± 16	424± 24			
	9	744± 31	495± 22	1,239± 29	715± 46	350± 32	1,065± 40
	CGR	58	33	91	55	17	72
Peanut	0	1,938±116	1,555±148	3,493±133			
	7	4,035±257	3,721±226	7,756±244	3,027±188	2,689±238	5,716±211
	CGR	230	309	539	156	162	318
Soybean	0	937±111	775±138	1,712±130			
	7	2,517±162	2,231±246	4,748±214	2,001±166	1,845±211	3,846±191
	CGR	226	208	434	152	153	305

[a]Fresh weight at 0 day was similar for both N treatments.

[b]Crop growth rate expressed in mg fresh weight per day in culture.

grew better in the ammonium than in the nitrate solution, whereas the opposite was true in root. Shoot fresh weight of nitrate treatment was only 87—89% of that of ammonium treatment and the value of 140—195% was recorded in root.

Crop growth rate (CGR, expressed in mg fresh weight gained per seedling per day) was also higher for the nitrate-grown plants than for the ammonium-grown plants (Table 1). Highest CGR was found in maize (1,307 mg for nitrate and 498 mg for ammonium treatment) followed by peanut and soybean. CGR of the small-grain crops, i. e., sorghum, rice, wheat and barley was lower when compared with othr crops tested.

Comparison between Tainung 67, a medium-grain rice variety, and Pegonil, a large-grain variety (grain weight of 25 and 40mg, respectively) showed no difference in response to different N sources. However, the large-grain Pegonil grew much faster than Tainung 67. After 13 days in culture, seedling fresh weights were 1,535-1,611 mg

for the former and only 865-930 mg for the latter. Again, CGR of of Pegonil was significantly higher than that of Tainung 67 (99-104 mg vs. 57-63mg).

During biochemical analysis of plants subjected to different N-source treatments, it was found that root PEP carboxylase was enhanced significantly by ammonium-N. Therefore, efforts have been made to explore its characters as well as its role in carbon metabolism as influenced by N supply. Table 2 showed PEP carboxylase activities in the leaf blade and root of various crops grown in either 150 ppm nitrate or 150 ppm ammonia. With the exception of wheat and barley, ammonium-N increased the activity of this enzyme in the leaf blade of the tested crop species although the increase was moderate. It was 8% for rice Tainung 67 and Taichung sen 3, 26—31 % for maize and sorghum and 61% for Pegonil rice. Absolute values, expressed in the unit of decrease in absorbance at 340 nm per gram fresh weight per minute, were highest for sorghum (262 for nitrate vs. 343 for ammonium-N) followed by maize (141 vs. 178). Rice had higher PEP carboxylase activities than both wheat and barley. On the other hand, PEP carboxylase activity in the leaf blade of wheat and barley decreased (32—40%) as a result of ammonium influence (Table 2).

Table 2. PEP carboxylase activities ($\triangle A_{340}$ gfw^{-1} min^{-1}) in the leaf blade and root of various crops as affected by different N sources

Crop	Days in Culture	Leaf Blade			Root		
		NO_3^-	NH_4^+	NH_4^+/NO_3	NO_3^-	NH_4^+	NH_4^+/NO_3
Cron	9	140.92	177.56	1.26	3.79	11.25	2.97
Sorghum	7	262.61	342.86	1.31	4.67	7.91	1.69
Rice Tainung 67	13	7.92	8.53	1.08	4.20	9.63	2.29
Rice Pegonil	13	13.65	21.98	1.61	3.95	8.41	2.13
Rice Taichung sen 3	15	9.34	10.11	1.08	5.92	10.78	1.82
Wheat	9	6.53	4.43	0.68	3.22	14.85	4.61
Barley	9	3.87	2.31	0.60	2.33	7.50	3.22
Peanut	7	nd[a]	nd	nd	0.52	2.49	4.79
Soybean	7	nd	nd	nd	3.03	2.77	0.91

[a]Not determined.

Very pronounced increases of PEP carboxylase activity were found in the root system of almost every crop tested (the only exception was soybean) as a function of ammonium treatment. The increases were from 3.79 to 11.25 units for maize (2.97-fold increase), 4.20 to 9.63 units for rice variety Tainung 67 (2.29-fold), 3.95 to 8.41 units for rice variety Pegonil (2.13-fold), 3.22 to 14.85 units for wheat (4.61-fold), 2.33 to 7.50 units for barley (3.22-fold) and 0.52 to 2.49 units for peanut (4.79-fold.) A slightly decrease in PEP carborylase activity was found in soybean grown in ammonium-N source. It was from 3.03 to 2.77 units (Table 2).

In order to verify that the increase of PEP carboxylase activity was a true response to ammonium-N, 10-day-old seedlings of Tainung 67 rice were transplanted from vermiculate (supplied with tap water only) to culture solutions containing nitrate-N (30 ppm) or ammonium-N of different concentrations (1, 10, 30 and 150 ppm) and PEP carboxylase activity in the root was determined with the advance of seedling growth for up to 15 days (Fig . 1). The initial value was 1.99 units. Activity increased continuously but slightly for plants grown in nitrate-N and was only 4.32 units at the 15th day of culture. For those plants grown in 1 ppm or 10 ppm ammonium-N, the increase in root PEP carboxylase activity was also continuous but at a higher rate. The acti-

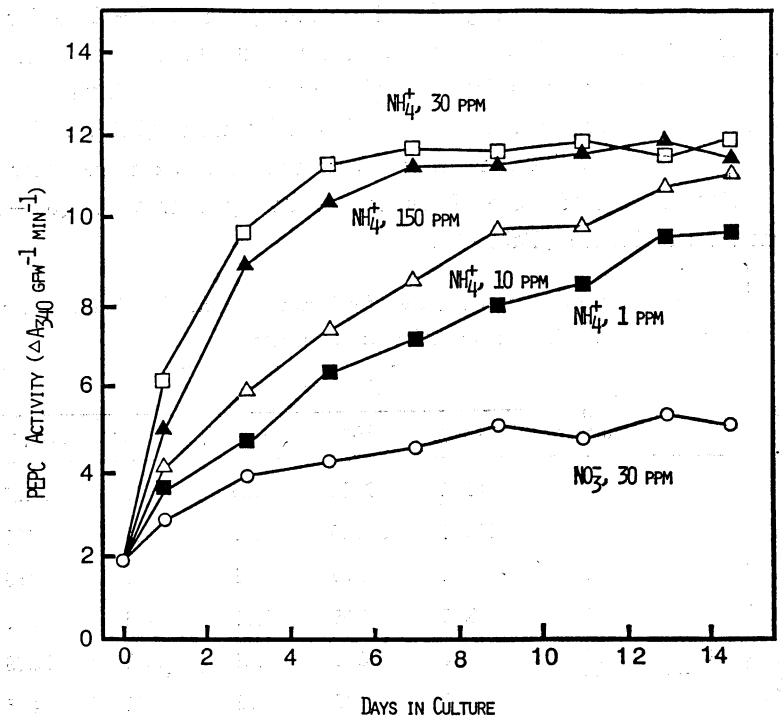

Fig. 1. Responses of root PEP carboxylase activities in developing Tainung 67 rice seedlings to different concentrations of NH_4^+ and NO_3^-.

vities at the 15th day were 9.14 and 10.87 units, respectively. Plants supplied with higher concentrations of ammonia (30 and 150 ppm) showed peak PEP carboxylase activities after 7 days in culture and the values were 2.6 times higher than that of the nitrate treatment. In other words, ammonium concentration of 30 ppm was high enough for the increase of PEP carboxylase activity in the root system of rice and the maximum response was obtained within one week after treatment.

Further studies were made to understand the nature of root PEP carboxylase enhanced by ammonium-N. Fig. 2 showed the diurnal changes of the enzyme activity of rice Tainung 67 grown in 150 ppm ammonium solution. Although there were fluctuations during the 24-hr cycle, the variations were less significant and the response to light

was less clear than those found in the leaf blades of many C4 plants. The activity remained fairly constant from 1 a. m. to 10 a.m. followed by a slightly decrease towards 2 p.m. It increased again until 10 p.m.and then decreased (day time or light time in the growth chamber was from 6 a.m. to 8 p.m.). Since there was no significant difference in PEP carboxylase activity from 8 to 10 a.m., samplings were made during this period for the following experiments in order to obtain results for comparison.

Fig. 2 also showed diurnal changes in soluble protein and malate concentrations in the rice roots. Changes in soluble protein concentration during the 24-hr cycle were in good accordance with those in PEP carboxylase activity. Statistical analysis showed a positive correlation between the two characters ($r=0.941^{**}$). Malate content in the

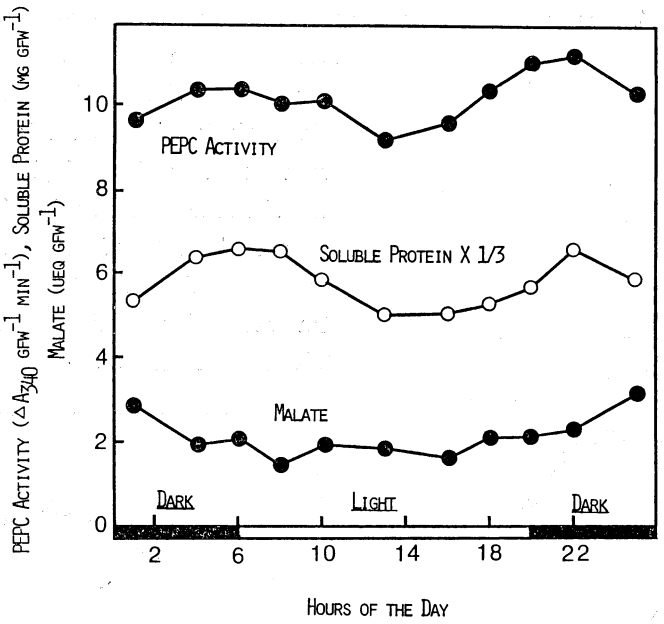

Fig. 2. Diurnal changes of PEP carboxylase activity and contents of soluble protein and malate in Tainung 67 rice roots. 15-day-old seedlings cultured in 150 ppm NH_4^+ solution were used. Data were means of 3-4 measurements.

rice roots was low and the values ranged from 1.72 to 2.86 micro-equivalent per gram fresh weight. There was a tendency that malate content was lower during the day time and higher during the night time although differences were not significant. No correlation was found between PEP carboxylase activities and malate during this diurnal study.

As reports on root PEP carboxylase were very limited, efforts have been made to study the characteristics of this enzyme in rice roots supplied with ammonium-N. Fig. 3A showed rice root PEP carboxylase was very stable with the method employed in this experiment. Activity remined fairly constant for up to 7 hrs after extraction. An 1-6% increase in enzyme activity was recorded during this period which was consi-

dered nonsignificant.

Fig. 3B introduced the responses of rice root PEP carboxylase activity to different pH values. With 100 mM Tris-HCl buffer, maximum enzyme activity was recorded at pH 8.5. The enzyme showed high activities within the pH range of 7.5 to 9.0. Activity decreased sharply towards acidic pH and rather moderately towards alkaline pH. As Hepes-KOH was used extensively in the study of PEP carboxylase by many scholars, it was also included in this experiment to test its effect in the range of 6.0 to 8.5. The results obtained supported those from Tris-HCl study. Maximum activity was recorded at pH 8.0 with slightly decrease in activities at pH 7.5 and 8.5. However, PEP carboxylase activity was much lower at pH 7.0 with Hepes-KOH than with Tris-HCl(Fig.3B).

A comparison was made on PEP carboxylase activity at pH 7 and 8 with varying concentrations of PEP. Response curves of velocity versus PEP concentration were presented in Fig. 4. The maximum activity was substantially higher at pH 8.0 than at 7.0. Response curve was hyperbolic in nature at pH 8.0 and sigmoid at pH 7.0. Kinetic analysis of the data in Fig. 4 was made from double-reciprocal plots of velocity versus substrate concentrations(data not shown). Reciprocal plots of the data were linear, indicating Michaelis-Menten Kinetics, and the Km value for PEP was calculated to be 0.16 mM at pH 8.0.

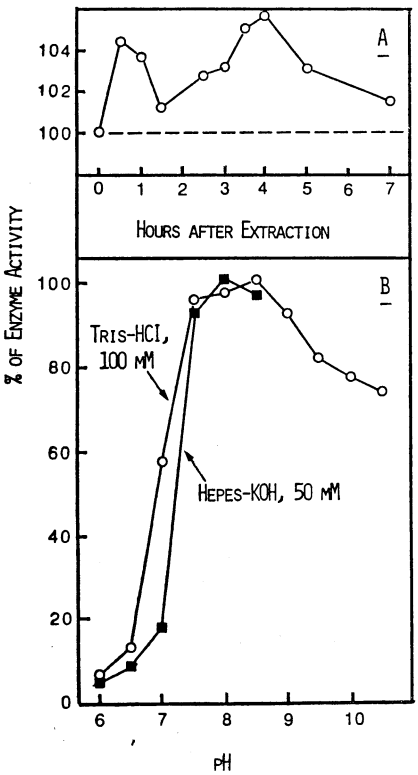

Fig. 3. Influences of (A) time elapsed after extraction and (B) pH value on the activity of PEP carboxylase in Tainung 67 rice roots. 15-day-old seedlings were used. Data were means of duplicate measurements.

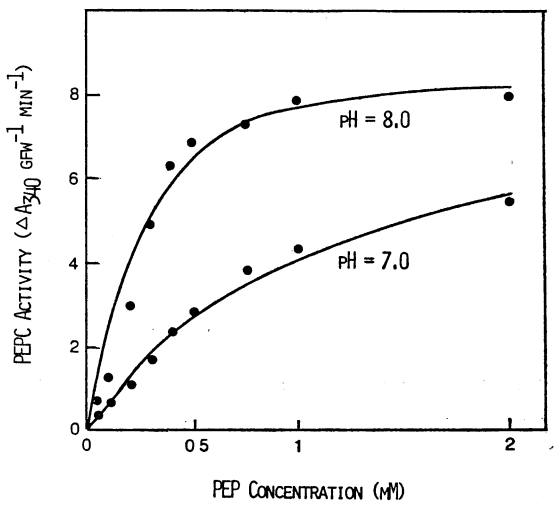

Fig. 4. Effects of substrate(phosphoenolpyruvate) concentrations on the PEP carboxylase activitz in Tainung 67 rice roots. 15-day-old seedlings were used. Data were means of duplicate measurements. Activities at concentrations of 5 and 10 mM decreased at both pH values.

Discussion

Culture medium containing the anionic nitrate became more alkaline and those with cationic ammonia more acidic with the advance of plant growth. The changes in external pH are due to excretion of hydrogen ions by the plant upon ammonium uptake, and hydroxyl ions upon nitrate uptake[22]. In general, lower pH was recorded with larger root volume and higher absorption capability of the plants. No significant difference among the seven crop species was found.

Weight gains by adding ammonia to an all nitrate system have been observed in many plant species[8]. Cox and Reisenauer[1] reported that growth rate and yield of wheat were enhanced under similar condition. The enhancement was suggested to result from the reduced energy requirement in using ammonia in protein synthesis. However, ammonia alone usually possesses a detrimental effect on plant growth. Results of this experiment showed that seedling growth (fresh weight gain) of almost all the crops was inferior when grown under ammonium nutrition (Table 1). Possible causes are the toxicity of ammonium ions to plant metabolism and the lowering of pH in culture medium.

It is well known that nutrient supply affects the biomass distribution in plants, especially with respect to the shoot/root ratio[5] which indicates a change in the effectiveness of shoot or root as sink for assimilates. Rosnitschek-Schimmel[23] reported that in *Urtica dioica*, low N supply favored dry matter formation of the root plus rhizome fraction, whereas high N supply resulted in a preferential growth of the leaf plus stalk fraction. In the current study, seedlings of maize, sorghum, peanut and soybean gained more fresh weight in both shoot and root when grown in nitrate than in ammonium medium. On the contrary, shoot of rice and wheat grew better in the ammonium than in the nitrate solution, whereas the opposite was true in roots (Table 1). It is therefore suggested that not only the extent of N supply but also the N source affect plant growth and N utilization and storage. Variation in responeses to N sources among different plant species was also suggested.

Stimulation of PEP carboxylase in the photosynthetic organs by ammonium N has been reported by De Benedetti *et al.*[4] and Paul *et al.*[19]. Howewer, negative effect of ammonia on the activity of this enzyme has also been realized[13,28]. Results from this study showed that in the leaf blades of maize, sorghum and rice at seedling stage, ammonium-N could increase the activity of PEP carboxylase by 8-61% (Table 2). The influence of such increase on photosynthetic rate remains to be verified. Similar effect was also observed in the roots of almost all the crop species studied. The degree of increasing enzyme activity ranged from 82% to 4.8 folds (Table 2). The hypothesis was further supported by growing rice seedlings in culture solutions containing different concentrations of ammonia (Fig. 1). It was concluded that ammonium concentration as low as 10 ppm colud effectively exhibit the stimulatory function. Results from culture in 1 ppm ammonium also showed distinct difference from those cultured in 30 ppm nitrate. The extraordinarily high activity is not commonly found in the root tissue of

most C3 and C4 plants. It is possible that a considerable amount of carbon dioxide must be fixed by the ammonium-grown plants because earlier report by Ku *et al.* [11] indicated a high total titratable acidity but a low malate content in such plants.

Little information is available on the PEP carboxylase from plant root tissues. In addition, Ting and Osmond [26] and O'Learly [17] stated that PEP carboxylase extracted from the roots of several C4 plants was distinct from the leaf forms. It is therefore necessary to study further the nature of this enzyme, especially under ammonium-N condition, if detailed investigations are to be made. No significant diurnal change in PEP carboxylase activity was found (Fig. 2) . This is understandable as in the dark condition, root PEP carboxylase might not respond to light as leaf PEP carboxylase does. The moderate fluctuation in enzyme activity during the 24-hr cycle corresponded well with the content of soluble protein in the root tissue. In other words, specific activity of this enzyme was fairly constant through the experimental period.

Malate content in the roots of ammonium-grown rice plants was low and was not in correlation with the activity of PEP carboxylase. In generall, oxalacetate formed in the cells can be reduced to malate catalyzed by NAD- or NADP-malate dehydrogenase or converted to aspartate by aspartate aminotransferase. The current experiment did not analyze the activities of these two enzymes. According to earlier and unpublished results of this laboratory, however, wheat plants treated with ammonium-N had extremely high NAD-malate dehydrogenase activity compared to the nitrate-grown plants. Whether this is true in rice is unknown, and the disagreement between malate content and related ezyme activities has yet to be clarified.

The stability of PEP carboxylase after extraction has drawn attentions from many plant biochemists. According to O'Leary [17], activity of the enzyme may change substantially after extraction, especially for CAM plants. For example, if PEP carboxylase in CAM plant leaves was assayed within 1-2 minutes after extraction, the enzyme showed large diurnal variations in activity. However, if extracts were allowed to stand for an hour or more prior to assay, these diurnal variations disappeared. Gavalas *et al.* [6] also indicated that PEP carboxylase in desalted leaf extracts suffered a fast inactivation when activity was assayed at low PEP levels (e. g., below 0.6 mM). Results in Fig. 3A of this experiment showed that PEP carboxylase extracted from rice root with the described method remained fairly constant for up to 7 hrs after extraction. One possible explanation is that 20% glycerol was included in the extraction medium which may stabilize the enzyme [7].

For the assay of PEP carboxylase activity, desalting of the crude extract on a small column of Sephadex G-25 has been practiced extensively as reported by O'Leary [17], Gavalas *et al.* [6] and Karabourniotis *et al.* [10]. Test of desalting on Sephadex G-25 column showed no difference in enzyme activity in this experiment and hence no desalting was practiced in the current study.

PEP carboxylase is usually assayed in the pH range of 7.0—8.5. Studies at single substrate concentration indicate that the enzyme has maximum activity near pH 8 [17]. The affinity of PEP for the maize leaf enzyme decreases above pH 9.5 and below

7.5 [18]. PEP carboxylase extracted from the root of rice plant grown in ammonium nutrition also showed a pH optimum at pH 8.0—8.5 with either Tris-HCl or Hepes-KOH as buffering material (Fig. 3B). This was also in good agreement with the results on C4 plants by Huber and Edwards [9].

At pH 8.0, maize leaf PEP carboxylase showed hyperbolic kinetics with a Km near 1 mM. At pH 7.0, however, the kinetics were sigmoid with a Km that was several fold higher than that at pH 8.0 [17]. The situation with regard to PEP carboxylase from other sources is less clear. In a limited number of reports, Miziorko *et al.* [14] found hyperbolic kinetics with respect to PEP in spinach leaves. Sigmoid kinetics have been reported for enzyme from cotton [15]. In the present study, it was found that for rice root PEP carboxylase, the kinetics were hyperbolic at pH 8.0 and near sigmoid at pH 7.0. Reciprocal plots of the data were linear, indicating Michaelis-Menten kinetics, and the Km value for PEP was calculated to be 0.16 mM at pH 8.0. O'Leary [17] stated that Km value for PEP were about five-fold lower on average for the enzyme from C3 plants than that from C4 plants. In studying the kinetics properties of PEP carboxylase from C3, C4 and C3/C4 intermediate *Flaveria* species, Nakamoto *et al.* [16] reported the response curves were hyperbolic for the C3 and C3/C4 species at either pH 7 or 8 while they were sigmoidal for the C4 species at pH 7 and hyperbolic at pH 8. The Km value for PEP determined from reciprocal plots was the lowest in the C-3 species. Based on the above experimental results and discussion, it is suggested that the ammonium-N resposive root PEP carboxylase from rice roots is similar to the same enzyme from leaf blades of C3 plants in many aspects. More experiments should be done in order to provide a complete understanding of this root enzyme. Results reported here can be utilized as basis for future study in this very important field of plant biochemistry.

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硝酸態與銨態氮源對作物幼苗生長與根部 PEP Carboxylase 活性之影響[1]

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摘　要

將水稻與玉米等七種作物培養於含有150 ppm 之硝酸態或銨態氮水耕液中，測定幼苗生長速率，又由於發現銨態氮可顯著提高作物根部 PEP carboxylase 活性，故以水稻爲材料，初步究明其特性，供爲深入探討氮源對以 PEP 爲中心之碳素代謝作用影響的依據。試驗結果顯示各種作物幼苗在含硝酸態氮之水耕液中的生長較爲良好，但水稻與小麥莖葉生長則以銨態氮處理爲佳，亦即作物間的反應具有差異。銨態氮可使各種作物幼苗葉片與根部 PEP carboxylase 活性升高，其幅度在葉片爲 8～61%，在根部最高可達 4.5 倍以上。由本試驗方法萃取所得臺農67號水稻根部 PEP carboxylase 具有極高的穩定性，萃取後 7 小時之活性仍未降低；其最適pH值在使用 Tris-HCl 或 Hepes-KOH 緩衝液時，均爲 8.0 至 8.5。對此一自水稻根部萃取之酵素的Kinetic特性試驗得知，針對 PEP 濃度之反應曲線在 pH 爲 8.0 係屬 hyperbolic ，在 pH 爲 7.0 係屬 sigmoid，經計算 pH 爲 8.0 時之 Km 值爲 0.16 mM。初步判定此一酵素與 C-3 型植物葉片內 PEP carboxylase 之特性相同。由於 PEP 在作物含碳化合物之代謝作用方面極具重要性，而極低之銨態氮濃度（1—10 ppm）即可提升根部 PEP carboxylase 酵素活性，故爲深討氮素對作物生理活性影響之良好資料。

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